USING SPECIFIC BINDING DNA CAPTURE ELEMENTS TO DIRECT PULSED POWER KILLING OF BIOLOGICAL AGENTS

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*Abstract**

We have demonstrated that coating bacterial spores with organic semiconductor (diazoluminomelanin) enhances killing of these spores by pulsed microwave energy in aqueous solutions. Here we present additional data showing that this can be done with dry agent in a pulsed corona plasma reactor. We also show that the reduction/oxidation reactions that mediate these effects can be generated on the immediate surface of the target spores by microwave pulses by linking the organic semiconductor specifically to the spore surface with a DNA capture element.

I. INTRODUCTION

Microwave induced breakdown, or cavitation, using organic semiconductor in solution (diazoluminomelanin, DALM) produces spectral emissions indicative of plasma generation [1-5]. To kill target biological agent (spores), using this breakdown mechanism, (spores) required relatively high power (2 MW) or long duration (15 minutes) exposures at lower powers (1 kW) [3-5]. Here we examine the possibility that specifically linking DALM to target biological agents (spores) with DNA capture elements can reduce the exposure time or power necessary for destruction of the target agents by pulsed power sources.

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II. METHODS

Methods for microwave-induced breakdown have been previously described [3-5]. In brief, a radar transmitter, operating at 2.06 GHz provided 10.6 ms pulses at 10 pulses per second at a forward power of 1 kW.

The pulse corona reactor employed for comparison to the pulsed microwave killing of dry spores was custom made by Titan Corporation [6]. In brief, it generated the following parameters: (1) variable gas (air) flow rate; (2) variable average operating power to 1kW; (3) maximum pulsed voltage of from 10 kV to 30 kV; (4) pulse repetition rate variable to 2000 Hz. The spores were dried onto plastic or metal pins placed in various locations within the device or in its exhaust gas path.

The data reported are from water, physiological phosphate buffered saline, and diazoluminomelanin (DALM) semiconductor solutions prepared as previously reported for microwave-induced breakdown [3-5]. The DALM was synthesized in the *E. coli* strain JM109/pIC2ORN1.1 (American Type Culture Collection #69905). This *E. coli*'s plasmid was further modified to contain DNA capture elements (DCE, aptamers) made specifically against *Bacillus anthracis* (Sterne strain) spores, with a limited amount of cross reactivity against *Bacillus thuringiensis v. kurstaki* spores. The methods are thoroughly described in two United States patents [7, 8].

III. RESULTS

Figures 1A and B show pulsed microwave effects on the recovery of viable spores from DNA/DCE-coated spores of *B. thuringiensis* and *B. anthracis*, respectively, exposed to 1 min 15 seconds to 5 mins of radiation.

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14. ABSTRACT
We have demonstrated that coating bacterial spores with organic semiconductor (diazoluminomelanin) enhances killing of these spores by pulsed microwave energy in aqueous solutions. Here we present additional data showing that this can be done with dry agent in a pulsed corona plasma reactor. We also show that the reduction/oxidation reactions that mediate these effects can be generated on the immediate surface of the target spores by microwave pulses by linking the organic semiconductor specifically to the spore surface with a DNA capture element.

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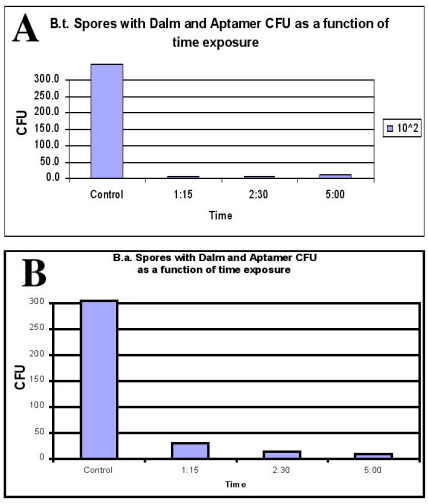

Figure 1A and B. Comparison of effects of pulsed microwave exposure (1 kW, 10 Hz, 10.6 ms pulses, 2.06 GHz) on viability of *Bacillus thuringiensis* (Bt; **A**) *Bacillus anthracis* (Ba, Sterne strain; **B**) spores (CFU= colony forming units).

Figure 2 shows the results of 1 min 15 sec exposures for various control and DNA/DCE-coated spores.

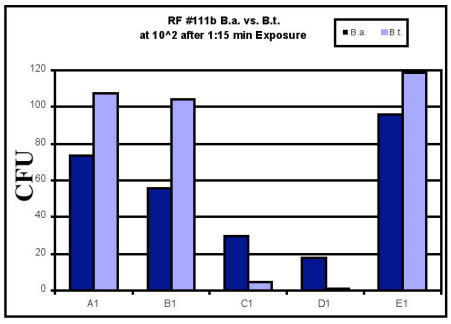

Figure 2. Comparison of viability of *Bacillus thuringiensis* (Bt) and *Bacillus anthracis* (Ba) spores following various treatments and pulsed microwave exposure. A= sodium bicarbonate and hydrogen peroxide; B= same plus biosynthetic DALM; C= same plus DALM/poly-valent DCE; D= same plus DALM/poly-valent DCE and more DCE plasmid; E= phosphate buffered saline.

Figure 3 shows the morphological effects of the corona plasma reactor and the brief pulsed microwave exposures.

IV. CONCLUSIONS

Based on the example data presented here, we conclude that when the time of exposure of high powered microwave pulses is decreased and the spores are coated with DNA capture elements linked to organic semiconductor, changes similar to the effects of cold plasma exposure are seen and the contents of spores are extruded. Furthermore, the specific linking of the organic semiconductor to the spores by DCEs leads to enhanced

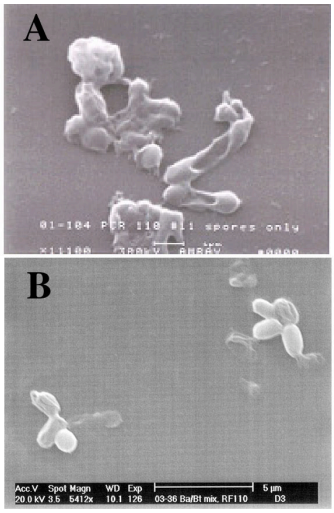

Figure 3. Scanning Electron micrograph comparison of pulsed corona plasma reactor (**A**) and pulsed microwave (**B**) effects on the morphology of spores.

killing of the spores at these decreased exposure times. These observations encourage further investigation toward linking the DCE–based detection and identification of biological agents with their destruction by plasma-generating modalities like pulsed corona discharge or microwave radiation.

V. DISCLAIMER

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army or Department of the Air Force position, policy, or decision unless so designated by other documentation.

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